

CLAIMS

- 5 1. An immunogenic composition comprising a recombinant protein and a polysaccharide component, wherein said protein is encoded by a gene from a strain of *Clostridium difficile* and said polysaccharide component is isolated from a strain of a pathogenic microorganism or chemically synthesized:
- 10 2. The immunogenic composition of claim 1, wherein said protein is a toxin or fragment thereof.
- 10 3. The immunogenic composition of claim 1, wherein said polysaccharide component is a capsular polysaccharide or a lipopolysaccharide.
- 15 4. The immunogenic composition of claim 1, wherein said protein is toxin A or a fragment thereof.
- 20 5. The immunogenic composition of claim 4, wherein said protein comprises a recombinant amino acid sequence that includes the toxin A repeating units (rARU) or a fragment thereof.
- 20 6. The immunogenic composition of claim 5, wherein said protein is a fusion protein.
- 25 7. The immunogenic composition of claim 1, wherein said protein is toxin B or a fragment thereof.
8. The immunogenic composition of claim 7, wherein said protein comprises a recombinant amino acid sequence that includes the toxin B repeating units (rBRU) or a fragment thereof.

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9. The immunogenic composition of claim 8, wherein said protein is a fusion protein.

5 10. The immunogenic composition of claim 1, wherein said immunogenic composition elicits in a mammalian host an immune response that is T-cell dependent.

10 11. The immunogenic composition of claim 1, wherein said immunogenic composition elicits in a mammalian host an immune response that is T-cell independent.

12. The immunogenic composition of claim 1, wherein said immunogenic composition elicits in a mammalian host an immune response that is both T-cell dependent and T-cell independent.

15 13. The immunogenic composition of claim 10 or 11 or 12, wherein said immune response is a cellular dependent immune response.

20 14. The immunogenic composition of claim 10 or 11 or 12, wherein said immune response results in a booster effect in said mammalian host.

25 15. The immunogenic composition of claim 10 or 11 or 12, wherein said immune response elicits a protective response to a strain of said pathogenic microorganism.

16. The immunogenic composition of claim 10 or 11 or 12, wherein said immunogenic composition elicits a humoral immune response in a mammalian host.

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17. The immunogenic composition of claim 10 or 11 or 12, wherein said immunogenic composition elicits both a humoral immune response and a cellular dependent immune response in a mammalian host.

5 18. The immunogenic composition of claim 10 or 11 or 12, wherein said immune response elicits a protective response to a strain of a pathogenic microorganism.

10 19. The immunogenic composition of claim 18, wherein said strain of a pathogenic microorganism produces said polysaccharide *in vivo*.

15 20. The immunogenic composition of claim 19, wherein said polysaccharide is isolated from a strain of a pathogenic microorganism selected from the group consisting of strains of: *Streptococcus pneumoniae*; *Neisseria meningitidis*; *Escherichia coli*; and *Shigella*.

20 21. The immunogenic composition of claim 20, wherein said immune response elicits a protective response to a strain of a pathogenic microorganism selected from the group consisting of strains of: *Streptococcus pneumoniae*; *Neisseria meningitidis*; *Escherichia coli* and *Shigella*.

25 22. The immunogenic composition of claim 19, wherein said polysaccharide is isolated from a serotype of *Streptococcus pneumoniae*, selected from the group consisting of serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 25, and 33F.

30 23. The immunogenic composition of claim 19, wherein said polysaccharide is isolated from serotype 14 of *Streptococcus pneumoniae*.

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24. The immunogenic composition of claim 18, wherein said immune response elicits a protective response to a strain of *Streptococcus pneumoniae*.

25. The immunogenic composition of claim 18, wherein said polysaccharide
5 is isolated from a strain of *Shigella flexneri*, serotype 2a.

26. The immunogenic composition of claim 18, wherein said immune response elicits a protective response to a strain of *Shigella*.

10 27. The immunogenic composition of claim 18, wherein said polysaccharide is isolated from *Escherichia coli* K1.

15 28. The immunogenic composition of claim 19, wherein said pathogenic microorganism is group B meningococcus (*Neisseria meningitidis* serogroup B).

29. The immunogenic composition of claim 19, wherein said pathogenic microorganism is *Escherichia coli* K1.

20 30. The immunogenic composition of claim 19, wherein said polysaccharide selected from the group of: *Staphylococcus aureus*; coagulase-negative *Staphylococcus*; *Enterococcus* species; *Enterobacter* species; *Candida* species; group B *Streptococcus*; *Escherichia coli*; and *Pseudomonas* species.

25 31. The immunogenic composition of claim 19, wherein said immune response elicits a protective response to a strain of a nosocomial pathogenic microorganism selected from the group consisting of strains of: *Staphylococcus aureus*; coagulase-negative *Staphylococcus*; *Enterococcus* species; *Enterobacter* species; *Candida* species; group B *Streptococcus*; *Escherichia coli*; and *Pseudomonas* species.

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32. The immunogenic composition of claim 19, wherein said polysaccharide is isolated from *Staphylococcus aureus* serogroup 5.

5 33. The immunogenic composition of claim 19, wherein said pathogenic microorganism is *Staphylococcus aureus* serogroup 5.

34. The immunogenic composition of claim 19, wherein said polysaccharide is isolated from *Staphylococcus aureus* serogroup 8.

10 35. The immunogenic composition of claim 19, wherein said pathogenic microorganism is *Staphylococcus aureus* serogroup 8.

15 36. An immunogenic composition comprising a recombinant protein and a polysaccharide component, wherein said protein is encoded by a gene isolated from a strain of *Clostridium difficile* and said polysaccharide is a polysaccharide isolated from a strain of a pathogenic microorganism or chemically synthesized and wherein said composition further comprises a pharmaceutically acceptable carrier.

20 37. A vaccine comprising the immunogenic composition of claim 36.

38. The vaccine of claim 37, wherein said vaccine is formulated for use in humans.

25 39. The vaccine of claim 37, wherein said vaccine is formulated for use in animals.

40. A method for producing an immunogenic composition, comprising constructing a genetic sequence encoding a recombinant protein, wherein said genetic sequence is isolated from a strain of *Clostridium difficile*;

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expressing said recombinant protein in a microbial host;
recovering said recombinant protein from a culture of said host;
conjugating said protein to a polysaccharide component, wherein said
polysaccharide component is isolated from a pathogenic microorganism or chemically
5 synthesized; and
recovering said conjugated protein and polysaccharide component.

41. The method of claim 40, wherein the expression of said genetic sequence
is regulated by an inducible promoter operatively positioned upstream of said sequence
10 and functional in said host.

42. The method of claim 40, wherein said microbial host is *Escherichia coli*.

43. The method of claim 42, wherein the recombinant protein is expressed at a
15 level greater than about 10 mg/ml.

44. The method of claim 42, wherein the recombinant protein is expressed at a
level greater than about 50 mg/liter of said culture.

45. The method of claim 42, wherein the recombinant protein is expressed at a
20 level greater than about 100 mg/liter of said culture.

46. The method of claim 40, wherein said protein is greater than about 50kDa.

47. The method of claim 40, wherein said protein is greater than about 90kDa.
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48. The method of claim 40, wherein said protein is recovered by ammonium
sulfate precipitation followed by ion exchange chromatography.

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49. The method of claim 40, wherein said protein is succinylated.

50. The method of claim 40, wherein said protein is conjugated to said polysaccharide component following a reaction of said protein and said polysaccharide component with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride.

51. The method of claim 40, wherein said polysaccharide component is activated by cyanogen bromide.

52. The method of claim 40, wherein said polysaccharide is derivatized by adipic acid dihydrazide.

53. A recombinant genetic sequence comprising a gene encoding a protein from a strain of *Clostridium difficile*.

54. The recombinant sequence of claim 53, wherein said gene encodes toxin A or a fragment thereof.

55. The recombinant sequence of claim 54, wherein said gene encodes the toxin A repeating units (rARU) or a fragment thereof.

56. The recombinant sequence of claim 53, wherein said gene encodes toxin B or a fragment thereof.

57. The recombinant sequence of claim 56, wherein said gene encodes the toxin B repeating units (rBRU) or a fragment thereof.

58. An expression vector comprising the genetic sequence of claim 53 and a gene that confers a selective phenotype upon a microbial host.

59. The expression vector of claim 58, wherein said selective phenotype is resistance to kanamycin.

5 60. A microbial host transformed with the expression vector of claim 58 or
claim 59.

61. The use of the immunogenic composition of claim 1 for the production of antibodies for passive immune therapy against a strain of said pathogenic microorganism.

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